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Author(s): Wong, Camille Hing

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Liquid Chromatography Mass Spectrometry Instrumentation and Methodology

Camille Wong

Engineered Materials Group (MST-7), Material Science and Technology Division
Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Abstract: This report reviews instrumentation and techniques utilized in liquid chromatography mass spectrometry (LC-MS). The analytical advantages and disadvantages of each major component of different LC-MS systems are discussed, along with a comparison between LC-MS, liquid chromatography tandem mass spectrometry (LC-MS/MS), and gas chromatography mass spectrometry (GC-MS). This report also details future work regarding the use of LC-MS/MS to analyze and identify degradation products from an extensive accelerated aging process of nitroplasticizer (NP). This report is the first of a series on the use of LC-MS to understand the degradation mechanisms of NP.

Introduction

Chromatography, a chemical separation technique, was originally developed for the separation of plant pigments in the very early 1900s. These pigments are composed of different colors, hence chrome, Greek for color. From the 1930s to 1950s, others types of chromatography were developed and made useful for many separation processes.[1] Separation techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) are based on separation based on several physical and chemical differences in analytes. The GC and LC techniques can range in selectivity and sensitivity but generally do not identify compounds. Therefore, the use of separation techniques in tandem with a detector, such as a mass spectrometer (MS) can overcome this limitation. Liquid chromatography mass spectrometry (LC-MS) covers a broad range of application areas. Over all, LC-MS refers to a tandem research analysis technique using chromatography as a separation technique followed by mass spectrometry to: sort, detect, and analyze molecular masses of analytes. Some LC-MS systems have two mass spectrometers in sequence and are referred to as liquid chromatography tandem mass spectrometry (LC-MS/MS). Tandem mass spectrometry (MS/MS) is a technique used to acquire molecular structural information by fragmenting analytes isolated in each MS analyzer. Better selectivity and sensitivity is achieved for quantitative analysis by selecting specific ion transitions between the first and second analyzers.[2] There are many different combinations of MS/MS systems, including tandem quadrupole time-of-flight (Q-TOF) and triple quadrupole, and ion traps.[3] There are also many different chromatography techniques that can be in sequence with mass spectrometers including HPLC and GC. Each different configuration of the LC-MS/MS system has its own strengths and limitations, this report reviews these instrumentations and analytical techniques.

The ultimate goal of this review is to help identify what is a suitable analytic technique to study the degradation products of nitroplasticizer (NP). NP is a type of plasticizer used together with polymer as a binder in many applications. Upon heating, NP degrades and generates reactive molecules, such as NO_x and water, which consequently degrade polymer.[4,5,6,7] The determination of the degraded fragment/molecules and their concentrations of aged NPs is a key step in understanding the reaction pathway and kinetics of NP degradation. This is a critical prerequisite for us eventually to be able to predict the long-term stability of NP/polymeric binders.[4] Therefore, it is important to select suitable chromatography technique for identifying known and unknown targeted compounds with high accuracy and sensitivity.

Gas and Liquid Chromatography

In the GC process of separation, a mixture is separated by exploiting differences in temperature required to transition molecules between liquid and gas states when adsorbed on a liquid or solid stationary phase. However, in LC the stationary phase is solid and the mobile phases are liquid.[1] The second difference between GC and LC is the column, through which the gas phase passes are located in an oven where temperature of the gas can be controlled. The columns in the LC are also located in an oven, but typically have temperature held at room temperature. Lastly, the concentration of the compound in the gas phase is a function of the vapor pressure of the gas due to Raoult's law.[1] Raoult's law states that the partial pressures of each component of an ideal mixture of liquids is equal to the vapor pressures of the pure component multiplied by its mole fraction in the mixture.[1]

GC uses a carrier gas as the mobile phase to transport sample components through either hollow or capillary type chromatographic columns.[8,9,10] The stationary phase inside the column is a microlayer of liquid or polymer on an inert solid support.[1] Traveling through the column, the gaseous compounds interact with the stationary phase which causes each compound to elute at different time resulting in different retention times. A comparison of the retention times from the

GC is then analyzed. Temperatures in a GC can exceed 350°C and may decompose thermally unstable analytes.[9] Normally, GC samples are prepared in organic solvents and analytes can be extracted from aqueous, organic, or solid samples. There are many types of GC detectors, the two most common ones are thermal conductivity detector (TCD) and flame ionization detector (FID).[1] TCD can detect any component with differing thermal conductivity from the carrier gas used and are non-destructive.[1] On the other hand, FID is destructive, resulting in the analyte not surviving the analysis intact.[8,9,10] Both TCD and FID are sensitive to a wide range of components and can work with a wide range of concentrations though FID cannot detect water.[1] The chromatographic separation in a GC column is mainly carried out on compounds with molecular weights up to a few hundred Daltons, because of volatility constraints.[8,9,10]

Liquid chromatography is a separation technique wherein the mobile phase is liquid, and the stationary phase is either composed of irregular or spherical shaped particles packed in a column.[8,10,11] There are low pressure (LC) and high-pressure liquid chromatography (HPLC) processes. LC relies on the force of gravity whereas HPLC has operational pressures from 50-350 bar.[1] HPLC analysis has no volatility issues, but the analyte must be soluble in the mobile phase.[8,10,11] This technique can analyze a wide range of analytes. The only restraint on an upper molecular weight limit is molecules being able to pass through the pores in the stationary phase. For these reasons, HPLC can separate from low molecular weight to many thousands of Daltons.[8,10,11] HPLC instruments typically include solvents, degasser, pumps, an auto-sampler, and a detector, Fig. 1. The sampler transports a sample into the mobile phase which then carries the sample to go through to the column. The pumps control the flow and composition of the mobile phase thought the column. The columns in HPLC are typically run at or near room temperature but can have a column oven for select separation techniques.[1] The retention mode of HPLC columns can depend on many different physical characteristics typically these are polarity differences, hydrophilic/phobic interactions, or molecule sizes; whereas GC operations are based on differences in volatilities of compounds.[9] HPLC has various detectors, including UV/Vis, photodiode array (PDA), and mass spectrometry. The detector generates a signal based on the retention times for the elute time from the column.[1] Overall, HPLC is a non-destructive separation technique unless the use of a destructive detector is utilized like MS.

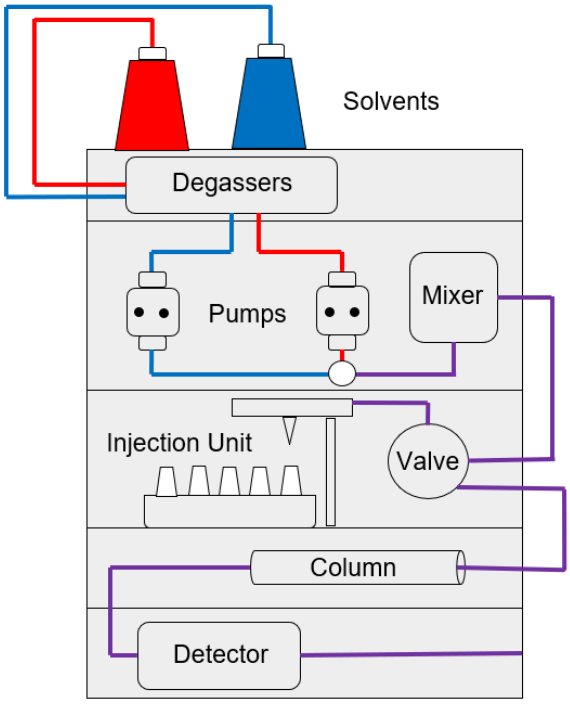

Figure 1. Schematic overview of an HPLC setup. The different colors represent the different solvents that run though the HPLC and the purple is a mixture of the different solvents. Modified from (Agüera, Fernández-Alba, Nishikawa, Katagi, Miki, Tsuchihashi).[8,10]

Mass Spectrometry

A mass spectrometer is an instrument designed to separate gas phase ions according to their mass to charge ratio (m/z). The separation of ions is achieved using electrical and/or magnetic fields to differentiate ions within the mass spectrometer. Every mass spectrometer includes an atmospheric ionization chamber, which is the interface between the ion source and the analyzer.[12] Each system also includes a detector and vacuum system that usually consist of a foreline pump as a rough vacuum with a high vacuum pump (or pumps) to establish the required high levels of vacuum.[15]

Ionization Source

Between the sample source and the MS, the eluent must change from liquid phase to gas phase, this is done in the ionization process. Once a sample elutes from the column, they are introduced into an Atmospheric Pressure Ion source (API) where they are converted into gas phase ions. Two typical ionization methods in LC-MS are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI),[13,4] which are different from the ionization methods used in GC-MS: electron ionization (EI) and chemical ionization (CI).[15,16]

Figure 2 shows a schematic of the ESI (top) and APCI (bottom) processes. ESI uses analyte desolvation and charge

transfer reaction in the gas phase to produce gas phase analyte ions. A charge potential is run across the eluent in the capillary tube and is then ionized before the sample leaves the capillary tube. The ions then directly interact with the interface or cone plate.[13,14] The principal difference in APCI and ESI is the charge potential in ESI occurs within the capillary tube, not in the desolvation zone as with APCI. ESI is commonly used with polar, ionized, and high molecular weight compounds.[13,14]

APCI uses liquid phase charge separation and ion evaporation techniques to produce vapor phase analyte ions. In APCI, liquid dispenses out of a capillary tip and then a separate probe (Corona needle) runs a charge potential across the liquid phase eluent.[13,17] A cloud of ionized molecules is formed in the desolvation zone prior to the electrospray interface or cone plate.[13,17] Using this ion source creates the formation of an ion cloud which can fragment the sample molecules prior to the fragmenting in the MS system.[13,14] APCI is suitable for polar and relatively less polar thermally stable compounds with molecular weight less than 1500 Da.[13,14] APCI allows high flow rates and reduces the thermal decomposition of the analyte because of the rapid desolvation and vaporization of the droplets in the initial stages.[16]

Both ESI and APCI are "soft" ionization methods meaning, in the process of ionization there is negligible energy transferred to the ion. Although, APCI is not as "soft" of an ionization technique as ESI which has low fragmentation.[17]

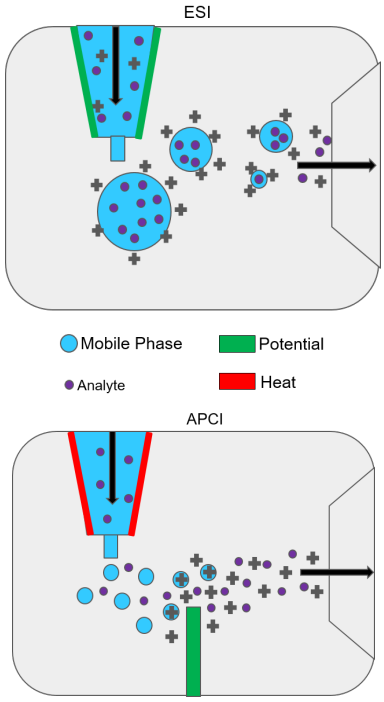

Figure 2. Schematic overview of two common ionization interfaces used in LC/MS. ESI (top), ionization on the liquid phase in capillary tube followed by desorption. APCI (bottom), vaporization by heat is followed by ionization of reagent molecules at the corona needle, then the ionized reagent molecules finally can transfer their charge to the target molecules. Modified from Verplaetse, Ruth & Tytgat, Jan.[17,18]

Electron ionization (EI) is an ionization method for GC-MS where analyte molecules are bombarded with free electrons emitted from a filament. The bombardment causes the molecules to fragment in a characteristics and reproducible way.[15] This technique is "hard" ionization which results in the creation of more fragments of low m/z. The molecule fragmentation patterns are dependent on the electron energy applied to the system, and are quite reproducible when the same fragmentation conditions are used.[15]

The use of chemical ionization (CI) in GC-MS requires reagent gas molecules are ionized by election ionization, which then react with analyte molecules in the gas phase in order to achieve ionization.[16] This type of ionization requires a lower amount of energy then EI, but is dependent on the reactant material. Due to low-energy ionization mechanism, there is little to no fragmentation. The lack of fragmentation limits the amount of structural information obtained about the ionized species.[16] However, even with less fragmentation the molecular weight of unknown analyte can be determined, resulting in a simpler and less detailed spectra then EI. Therefore this method is restricted to volatile compounds.[16]

Quadrupole

The quadrupole (Q) mass analyzer combines DC and RF potentials on the quadrupole rods which can be set to pass ions of a selected m/z.[19,20] The electric fields are used to separate ions according to the m/z as they pass along the central axis of four parallel equidistant rods. Ion separation uses DC and RF potential to control voltages applied to the rods which impart an electrostatic field.[19,20] When ions can pass through the quadrupole without touching the rods, this is known as non-collisional or stable trajectory. When the ion is caused to oscillate with an amplitude greater than the stable trajectory and the ion will collide with the rod and become discharged and subsequently pumped to waste, this is known as an unstable trajectory.[19,20] The none selected ions do not have a stable trajectory through the quadrupole mass analyzer and will collide with the rods. Fig. 3 demonstrates the paths of a stable and unstable trajectory. The collisions can cause fragmentation of the molecules or become discharged, therefore never reaching the detector.[11,19,20] Increasing the resolution decreases number of ions which reach the detector, due to resolution being proportional to frequency and length of quadrupole.[19] There are techniques that use multiple quadrupoles, hybrids, and variations. For example there are triple quadrupoles in linear series, electron sorting ion traps, and octopoles.

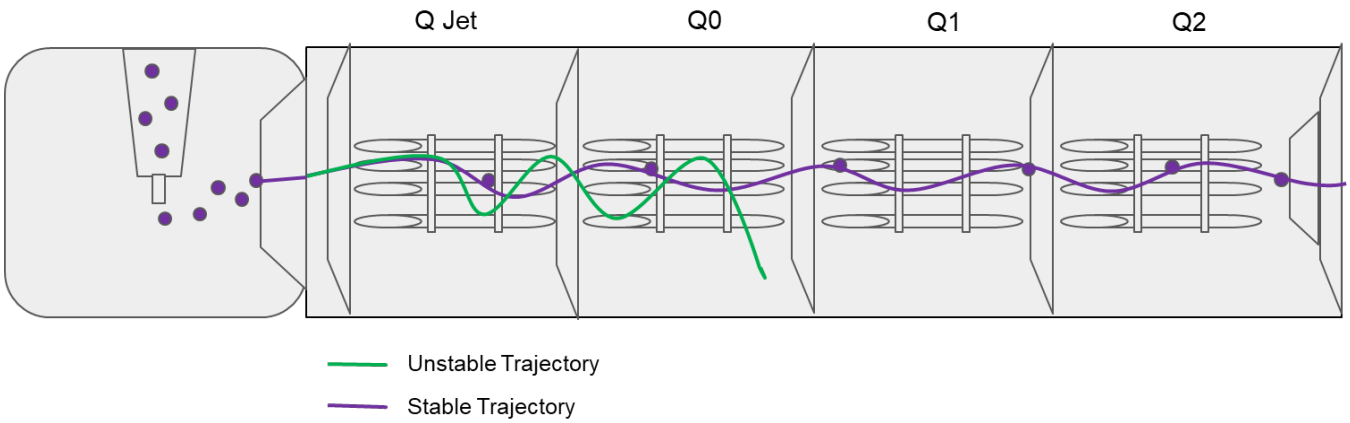

Figure 3. Scheme of a quadrupole mass analyzer with the stable and unstable trajectory. Only ions with selected mass-to-charge ratio pass the combined DC and RF potentials in the quadrupole rods.

Ion Trap

The Ion Trap or Trapped-Ion Mass Analyzers has four configurations of mass analyzes. There are three-directional quadrupole ion taps which are "dynamic" traps, ion cyclotron resonance mass spectrometers which are "static" traps, linear ion traps which are two dimensional, and electrostatic traps also known as Orbitrap.[19] The three-directional quadrupole ion traps or 3D ion trap operate by storing ions in the trap and manipulating the ions by using DC and RF electron field in a series of carefully timed events. In these geometries hardware is configured differently, where the parallel rods are replaced with two hyperbolic metal electrodes (end caps) facing each other, and a ring electrode placed halfway between the end cap electrodes; ions are trapped in a circular flight path based on the applied electric field.[3] The linear ion trap uses a set of quadrupole rods coupled with electrodes on each end to facilitate the ion trapping.[3] This configuration gives the linear ion trap a dual functionality, therefore it can be used as a quadrupole mass filter or an ion trap. The Orbitrap mass spectrometer consists of an inner spindle-like electrode and an outer barrel-like electrode. The Orbitrap stores ions in a stable flight path that orbit around the inner spindle by balancing their electrostatic attraction from their inertia coming from an RF only trap.[3,19] The frequency of the axial motion around the inner electrode are related to the m/z of the ion.[3]

Ion cyclotron resonance (ICR) traps use a strong magnetic field to induce a radial orbit of ions.[3] The frequency of radial orbit in the magnetic field is a function m/z for the ion. This allows several unique capabilities, such as extended MS/MS experiments, very high resolution, and high sensitivity. A disadvantage is trapping ions for long periods, ranging from milliseconds to hours, can be enough time for the ions to degrade spontaneously causing unimolecular decomposition.[19] Another disadvantage is unwanted interactions with other ions because space charge effect can occur, as well as the formation of neutral molecules from ion-molecule reactions and perturbation in the ion motion due to imperfect electric fields.[19] All these characteristics can lead to artifacts and unexpended changes in the mass spectrum.

Time-of-Flight

The Time-of-Flight (TOF) mass spectrometer measures the mass-dependent time. It takes ions of different mass to charge ratio (m/z) to move from the ion source to the detector.[23] Ions are extracted in short burst from the ion source and subjected to an acceleration voltage or ion puller.[21] The ions fly down an evacuated path, in a linear or non-linear geometry.[21] The use of reflection with ion mirrors allows the ions to fly down a non-linear path geometry.[21] Once free from the accelerating voltage region, the speed at which the ions fly in the path depends on the mass and charge.[11,19,20,23] TOF can detect all ions almost simultaneously while scanning the mass range of all ions very rapidly, therefore high sensitivity. This requires the ions leaving the ion source be well defined, and have different kinetic energies. However, many types of TOF have been developed to compensate for analytes which are difficult to decipher from one-another.[11,19,20] There are many different levels of resolution in a TOF MS which have a direct correlation with the geometry of the ion path within the instrument. The process to increase resolution is by increasing the flight length and minimizing contributions to flight time aberrations.[21] In order to accomplish these, changes in reflectrons and multi-pass flight paths are utilized. The common geometries are V- geometry with one reflectron, N- geometry with two reflectrons, and W-geometry with three reflectrons.[21] Fig. 4 is a representation of an N-geometry TOF.

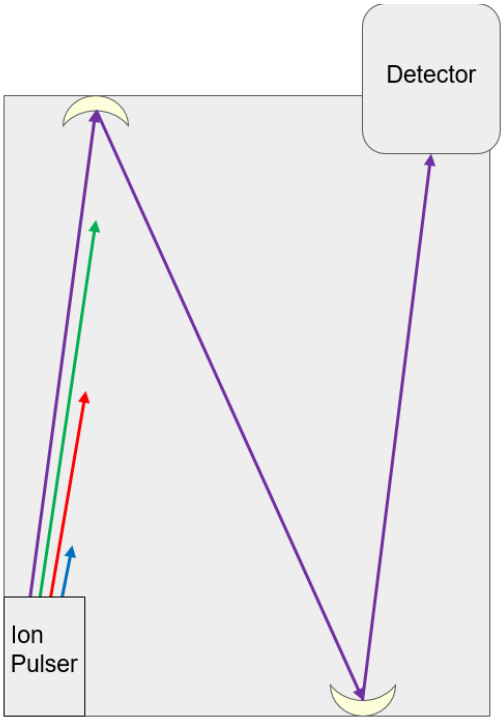

Figure 4. N-optic design/geometry with heated TOF path beginning with an ion pulser and ending with a detector. Colored arrows represent different ions of different m/z flying through the TOF.

The TOF instrument represents a powerful tool for identifying non-target compounds in complex environmental matrices because of three important characteristics.[11,19,20] First, TOF's ability to collect data across a wide mass range without a decrease in sensitivity. Therefore full spectral sensitivity is achieved. Second, high resolving power is also important and TOF's ability to resolve background from signal is enhanced by its ability to separate ions of similar flight times into separate signals.[11,19,20] Lastly, mass-measurement accuracy is high enough to determine elemental composition of a molecule within 5ppm due to mass defect, which is the difference between the exact mass and integer mass of a nuclide.[22]

Pros and Cons of GC-MS and LC-MS for Nitroplasticizer Characterization

The use of a separation technique with mass spectrometry enables an operator to acquire high levels of sensitivity needed for the study of NP degradation products. The use of one MS coupled with different separation techniques is first considered.

The advantages of the GC-MS based methods are: the high amount of structural information yielded and the possibility of using commercial libraries which make the identification of unknowns; the ruggedness and reliability of the GC-MS interface; and the highly sensitive and separation efficiency which avoid the over-lapping of compounds with similar structure.[10,11] This method does have drawbacks due to their low capacity for analyzing very polar, less volatile, thermally unstable compounds and has a range molecular weight in the several hundred Daltons. There are approaches in GC-MS that can use high polarity columns, but they are even more limited is there stability and bleeding at high temperature.[10,11]

For liquid samples, such as NP, the use of LC-MS presents several advantages over GC-MS. LC-MS requires little or no sample extraction and has the capacity to easily analyze highly polar, less volatile, thermally stable compounds. Furthermore, LC-MS has the ability to analyze a molecular weight range much larger than GC-MS. LC-MS also comes with the added benefit of direct analysis of the samples, avoiding the loss of volatile molecules escaping during extraction procedures.[8,24] However, the differences in chromatography techniques are not the only determining factor in a LC-MS system, between the different types of MS techniques drastically diverse results can exist. Considering the statements above, the separation technique best suited for the study of the NP degradation products is HPLC, because the samples have molecular weights of up to several hundreds of Daltons and are not thermally stable. Following the separation techniques in a LC-MS system is the ionization source. Each ionization source described is previous sections has their own advantages and disadvantages. There are the hard ionization techniques such as EI, which employ of molecular electron bombardment.[15] Then there are the soft ionization techniques such as ACPI, CI and ESI, which charge by molecular collision with an introduced gas.[15] Between the ionization sources discussed above, the use of soft ionization particularly the ESI process fits the NP samples.

In addition to the named small molecules (NO_x and water), there are many degraded fragments found in the aged NPs, which are proposed, but not experimentally identified.[5,6] Therefore, it is believed the Q-TOF MS best fits the requirements. The unique capability of the Q-TOF MS, as compared to the triple quadrupole and ion-trap MS/MS instruments lies in its ability to determine accurate mass on the fragment ions generated in the collision cell with in the quadrupole combined with the simultaneous high resolution and mass-measurement accuracy the TOF provides. Fig. 5 illustrates the setup for an HPLC Q-TOF MS system equipped in our group. Quadrupole MS allows ions of nominal mass to pass through the rods and there may be masses that interfere with the determination of the molecular ion.[11,19,20] Interfering ions are much less likely for the fragment ions, which in turn helps in the determination of accurate mass by lowering mass interferences and increasing accuracy with the same resolving power.[11,19,20] Quadrupole mass analyzers are employed in many LC-MS systems because of their relatively low cost, stable operation, and ion selectivity. A limitation of quadrupoles is their comparatively low resolution and mass discrimination.[19] In a TOF MS a curved-field reflection ensures the ideal detector position, so it does not vary with mass-to-charge ratio and this also improves resolution.[11,19,20] TOF analyzers are the fastest

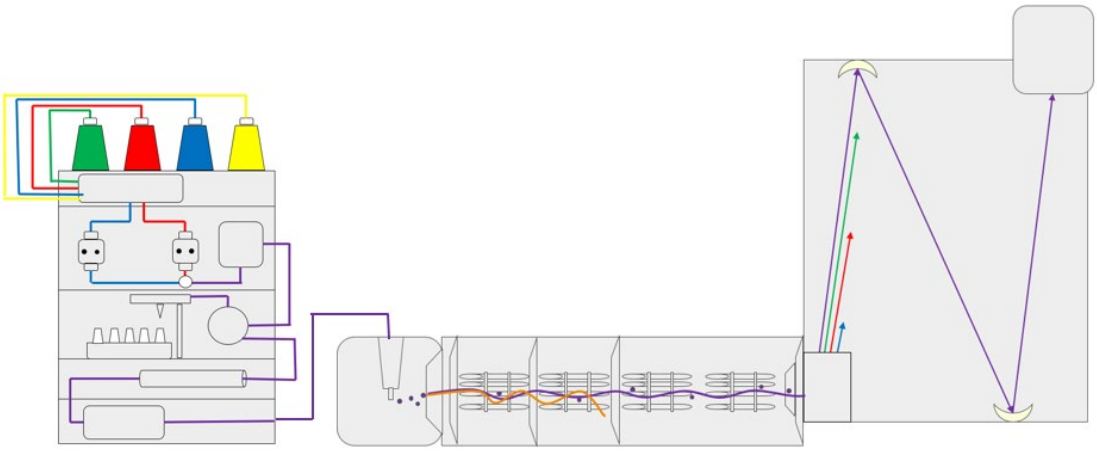

Figure 5. HPLC-QTOF system beginning with HPLC then ACPI or ESI ionization with a quadrupole MS followed by an N-geometry TOF MS ending with a detector.

MS analyzers and provide highly detailed spectra, making them suitable for application in high-performance LC-MS/MS. A limitation with a single TOF MS is limited ion selectivity. Therefore, a TOF coupled with a quadrupole allows for the best possible ion selectivity.[19] The use of quadrupole and TOF together are complimentary to each other's limitations. This results in identification of known and unknown compounds with high selectivity and accuracy comprised of full-scan sensitivity in a dynamic range.[11,20] Where the triple quadrupole, ion trap, and TOF MS fall short in one or more of these qualities on their own.

Summary and Future Work

The NP ageing project for which this review was conducted has a need for the identification on unknown and known compounds. In order to select the appropriate combination of techniques, the limitation and advantages of GC-MS and LC-MS techniques where explored and discussed. After discussing the possible combinations of separation, ionization and mass analyzer, the use of a HPLC with tandem quadrupole time-of-flight system best fits the requirements. HPLC-QTOF allows a high molecular weight range in the several thousands of Daltons along with ion fragmentation, ion selectivity, high resolution and mass-measurement accuracy required for identification. Eventually with the use of the HPLC-QTOF MS, the data collected will lead to the understanding of the kinetic mechanism of formation of degradation products and intermediates during aged NP degradation.

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Abbreviations

LC-MS, liquid chromatography mass spectrometry; LC-MS/MS, liquid chromatography tandem mass spectrometry; GC-MS, gas chromatography mass spectrometry; NP, nitroplasticizer; GC, gas chromatography; HPLC, high-performance liquid chromatography; MS/MS, Tandem mass spectrometry; Q-TOF, quadrupole time-of-flight; TCD, thermal conductivity detector; FID, flame ionization detector; LC, liquid chromatography; API, atmospheric pressure ion source; ESI, electrospray ionization; APCI, atmospheric pressure chemical ionization; EI, electron ionization; CI, chemical ionization; Q, quadrupole mass analyzer; ICR, ion cyclotron resonance; TOF, Time-of-Flight; m/z, mass to charge ratio.

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